Abstract

Point mutations in the mitochondrial DNA (mtDNA) result in the formation of defective proteins and thus in respiratory chain deficiencies, which cause heterogeneous disorders in humans. It was believed that mutated proteins are not assembled into respiratory chain complexes and are degraded by inner membrane quality control proteases. To understand the mechanisms of selective protein degradation and of mitochondrial stress response in more detail, a cybrid cell line containing a G6930A point mutation in the cytochrome c oxidase subunit 1 (COX1) of complex IV was studied. The mutation causes the formation of a premature stop-codon and thus a 30 % truncated protein is built, which however does not accumulate to steady state levels. G6930A cybrid cells not only show a complex IV defect, but the activities and steady state levels of respiratory chain complexes I, II and III are also impaired.

To investigate the nuclear response to mitochondrial stress, gene expression analysis (mRNA-Array) was performed, which revealed a decreased expression of many nuclear genes encoding respiratory chain subunits, excluding a compensatory upregulation of mitochondrial biogenesis. In addition mRNAs and proteins of the mitochondrial quality control proteases AFG3L2 and YME1L1 were upregulated. To investigate the role and the effect of the protease AFG3L2 in the degradation of respiratory chain (RC) subunits, pulse chase experiments were performed after transient transfection of AFG3L2 variants and after transient knockdown of AFG3L2. Overexpression of wild type AFG3L2 resulted in a higher degradation rate of RC subunits, whereas the overexpression of a dominant negative AFG3L2 variant ($AFG3L2^{E408Q}$) and the knockdown led to increased stability of RC subunits, demonstrating the crucial role of AFG3L2 in the degradation of mitochondrially encoded subunits. Furthermore the truncated COX1 protein as well as other respiratory chain subunits of complex I, IV and V were identified as substrates of AFG3L2 by pull-down experiments.

The data suggest a crosstalk between mitochondria and the nucleus to prevent accumulation of misfolded and unassembled respiratory chain proteins, thus preventing their possibly harmful effect in mitochondria. This objective is achieved on the one hand by downregulating gene expression of nuclear encoded respiratory chain subunits and on the other hand by upregulating mitochondrial quality control proteases.